## Appendix B

## Method for Estimating the Magnitude of Chronic Effects of Cyanide on Listed Species

The first preference for quantitatively assessing chronic effects of cyanide on listed species would be to use data from chronic toxicity tests/studies with the species in question. If, however, chronic toxicity data are not available or are not suitable it may be necessary to estimate effects on listed species using surrogate species. For cyanide, species-specific chronic toxicity data are not available for the listed species for which "likely to adversely effect" (LAA) determinations have been made. To estimate the type and magnitude of effects for evaluation in the Biological Opinion (BO), some estimate of the chronic effects of cyanide on LAA listed species is needed. The approach taken here was adapted from the screening level assessment employed in the BE methodology. According to the BE methodology, if chronic data for the listed species are not available the chronic threshold for the listed species, i.e. the "Assessment Effects Concentration" or EC<sub>A</sub> is estimated using data from surrogate species. The EC<sub>A</sub> is intended to estimate the highest chemical concentration in water (aquatic species) or food (aquatic-dependent species) that would cause no adverse effect or would adversely affect an acceptably-small percentage of individuals within a specified species population. For chronic toxicity, the ECA is based on the acute toxicity to the listed species, and the Acute to Chronic Ratio (ACR) of surrogate species. The ACR, for the purposes of the national BE methodology and here, is calculated as follows:

$$ACR = \frac{SS LC_{50}}{SS NOEC}$$
(1)

Where: SS LC<sub>50</sub> is the LC<sub>50</sub> for the surrogate species

SS NOEC is the No Observable Effects Concentration for the surrogate species

EC<sub>A</sub>'s are estimated using the following equation:

$$EC_{A} = \frac{LS LC_{50}}{ACR}$$
(2)

Where: LS  $LC_{50}$  is the  $LC_{50}$  for the listed species

The ACR and EC<sub>A</sub> are graphically illustrated in Figures 1 and 2. The effects determination for the BE is based on a comparison of the EC<sub>A</sub> for a listed species and the Criterion Continuous Concentration (CCC) for cyanide, 5.2 ug CN/L. If the EC<sub>A</sub> is less than 5.2 ug CN/L the species is likely to be adversely affected and if the EC<sub>A</sub> is greater than 5.2 ug CN/L the species is not likely to be adversely affected. The type of effect and its severity

(beyond likely versus not likely) were not part of the BE, but that information is needed for the BO to help characterize the magnitude of effects to individuals, populations and the species as a whole. As mentioned above, the BE method was modified in order to estimate the magnitude of effect that would occur if the listed species was exposed to cyanide at the CCC.

Equations 1 and 2 can be combined by substituting equation 1 for the ACR term in equation 2:

$$EC_A = \begin{array}{c} LS \ LC_{50} \\ SS \ LC_{50} \\ \hline \\ SS \ NOEC \end{array}$$

Rearrange:

$$EC_A = \frac{LS \ LC_{50}}{SS \ LC_{50}} * SS \ NOEC$$
 (3)

Figure 3 illustrates how equation 3 can also be used to calculate the  $EC_{A.}$  In this case the relative difference in sensitivity between listed species and surrogate species to acute exposures is used as a sensitivity adjustment factor to calculate the Chronic  $EC_{A}$  for the listed species from the surrogate species NOEC.

The level of effect occurring at the  $EC_A$  corresponds to the level of effect occurring at the surrogate species NOEC, and is intended to be acceptably low. However, the level of effect at the NOEC can vary between studies (refer to NOEC/LOEC discussion in the Cyanide BO) and thus the level of effect at the  $EC_A$  would vary as well. To more accurately reflect the level of effect associated with the  $EC_A$ , equation 3 can be rewritten in a more general form:

Where:

 $LS\ EC_X$  is the Effects Concentration for the listed species that elicits a response of magnitude X, and;

 $SS\ EC_X$  is the Effects Concentration for the surrogate species that elicits a response of magnitude X.

For the Biological Opinion, we are interested in estimating how listed species are affected by cyanide when exposed at the CCC (5.2 ug CN/L). We can use equation 4 to do this by first setting the LS EC<sub>X</sub> equal to the 5.2 ug CN/L then calculating the Effects Concentration

for the surrogate species, SS  $EC_X$ , and finally estimating X (magnitude of effect) from the exposure – response relationship for the surrogate species:

First, set

LS 
$$EC_X = 5.2 \text{ ug CN/L}$$

Substitute in equation 4,

$$5.2~ug~CN/L \quad = \begin{array}{c} LS~LC_{50} \\ ----- & *~SS~EC_X \\ SS~LC_{50} \end{array}$$

Next, rearrange:

$$SS EC_{X} = \frac{SS LC_{50}}{LS LC_{50}} * 5.2 ug CN/L$$

$$LS LC_{50}$$
(5)

Because the SS LC<sub>50</sub> and LS LC<sub>50</sub> are known (or estimated), setting the LS EC<sub>X</sub> equal to the 5.2 ug CN/L allows for the calculation of SS EC<sub>X</sub>. The SS EC<sub>X</sub> is the effects concentration for the surrogate species that is equivalent to the effects concentration for the listed species at the 5.2 ug CN/L, after adjusting for differences in sensitivity between the surrogate and listed species based on the ratio of acute toxicities, i.e. SS LC<sub>50</sub>/LS LC<sub>50</sub>.

For example, the  $LC_{50}$  for fathead minnow, a surrogate species, is 138 ug CN/L and the estimated  $LC_{50}$  for the Maryland darter, a listed species, is 40 ug CN/L. Based on these values fathead minnows would be 3.45 times less sensitive than Maryland darters and the SS  $EC_X$  would be 17.9 ug CN/L, that is, 3.45 times higher than the CCC (5.2 ug CN/L). In other words, fathead minnows would have to be exposed to a concentration 3.45 times higher than the CCC to experience an effect, equal in magnitude, to the effect on the Maryland darter exposed at the CCC.

Once the SS EC<sub>x</sub> is calculated, the magnitude of effect (X) can be estimated using the chronic exposure-response curve for the surrogate species (Figure 4). In this illustration the chronic toxicity data for the fathead minnow was fitted to a log-linear regression model. The magnitude of effect, X, at the SS EC<sub>x</sub> (17.9 ug CN/L) can be estimated using this model. For fathead minnow, an SS EC<sub>x</sub> of 17.9 ug CN/L corresponds to an effect of about 54%. Therefore, the CCC (5.2 ug CN/L) would correspond to an EC<sub>54</sub> for the Maryland darter, or a 54% effect concentration.

This estimation method relies on two underlying assumptions: (1) that relative differences in sensitivity between surrogate species and listed species to acute exposures are good approximations of the relative differences in sensitivity to chronic exposures and (2) that the slope of the exposure-response curves for surrogate and listed species are reasonably similar.

Figure 1. Illustration of how the Acute to Chronic Ratio (ACR) is determined.

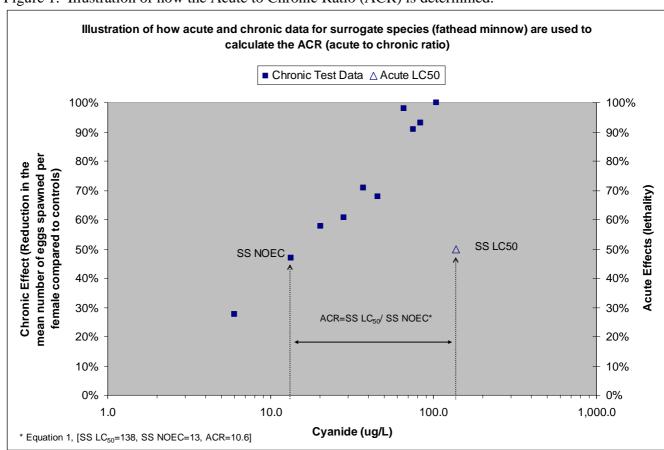


Figure 2. Illustration of how the ACR (Acute to Chronic Ratio) is used to estimate Chronic Assessment Effects concentrations ( $EC_A$ ).

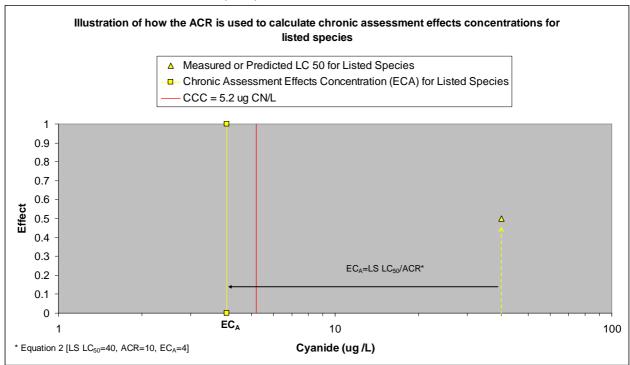


Figure 3. Illustration of how the Chronic Assessment Effects concentrations (EC<sub>A</sub>) may be estimated using the surrogate species NOEC and the ratio of the surrogate species  $LC_{50}$  to the listed species  $LC_{50}$ .

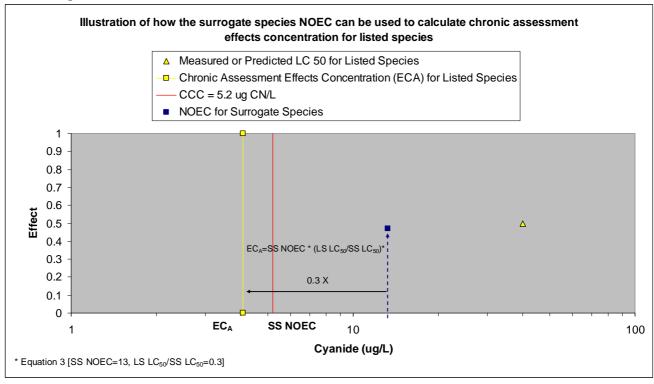


Figure 4. Illustration of how the magnitude of effect may be estimated using exposure – response data for surrogate species.

